Safety Data Sheet

Amatoxins

Division of Safety
National Institutes
of Health

WARNING!

THESE COMPOUNDS ARE ACUTELY TOXIC. THEY ARE READILY ABSORBED THROUGH THE INTESTINAL TRACT. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

HANDLE WITH EXTREME CARE. FOR INGESTION, REFER TO PHYSICIAN IMMEDIATELY.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. USE WATER TO DISSOLVE COMPOUND. WASH DOWN AREA WITH SOAP AND WATER. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

Amatoxins are a group of bicyclic octapeptides which constitute the most toxic components of the mushroon Amanita phalloides Secr. (syn. Green Death Cap, "gruner Knollenblatterpilz"). This particular species is mainly confined to Europe where most of the investigative work on isolation, structures, and mechanism of toxic action has been carried out (particularly in the laboratory of Th. Wieland); however, some true A. phalloides samples have been found in California, Oregon, and Washington (Tyler et al., 1966). Other American species containing amatoxins are A. bisporigera and A. suballiacea, the latter species being abundant in Florida (Little and Preston, 1984). The toxicities of amatoxins are of the order of 0.1-0.5 mg/kg (great species variation), and it is

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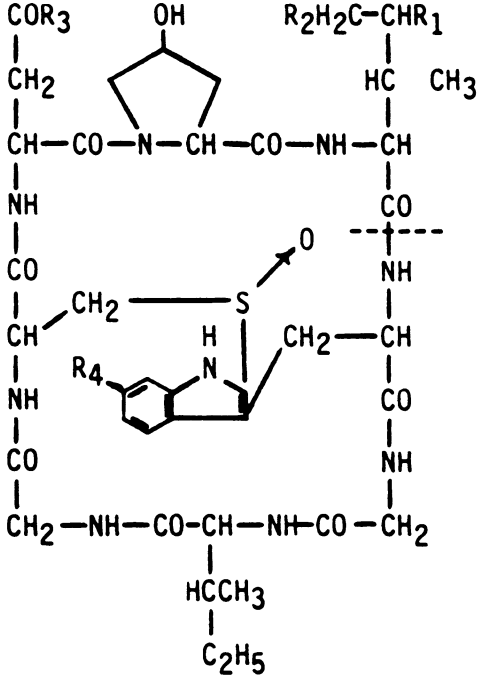
estimated that eating one toxic mushroom (40-50 g) can be lethal to man. The chief toxic target is liver and/or kidney (dependent on animal species and dose) where the toxins inhibit specifically the enzyme RNA polymerase II, thus preventing the formation of new messenger RNA, DNA, and protein. The main uses of amatoxins are in research on RNA and DNA formation, particularly in viral systems.

A second main group of Amanita toxins are phallotoxins, cyclic hept peptides whose toxicities are 10-50 times lower than those of amato cins. Representative phallotoxins are: phalloidin, phalloin, phallacidin, and phallisin. The onset of symptoms due to phallotox poisoning is much faster than those due to amatoxins, and most of t early symptoms (0.5 - 2 days) following Amanita ingestion are due t phallotoxins. They will not be considered in any detail in this da sheet.

Numerous recent reviews of amatoxins (and other toxic constituents of Amanita) have been published (e.g., Chilton, 1978; Lampe, 1978; Wieland and Faulstich, 1978; Wieland, 1983).

Chemical and Physical Data

1. Chemical structures, molecular formulas and Chemical Abstract numbers:



Name	R_1	R_2	R_3	R_4	Mol. formula	C.A. Number
α -amanitin	OH	OH	NH_2	OH	$C_{39}H_{54}N_{10}O_{14}S$	23109-05-9
β -amanitin	OH	OH	OH	OH	$C_{39}H_{53}N_9O_{15}S$	13567-07-2 21150-22-1
γ -amanitin	OH	H	NH_2	OH	$C_{39}H_{54}N_{10}O_{13}S$	13567-11-8 21150-23-2
δ -amanitin*						57107-61-6
ε -amanitin	OH	H	OH	OH	$C_{39}H_{53}N_9O_{14}S$	21705-02-2
amanin	OH	OH	OH	H	$C_{39}N_{53}N_9O_{14}S$	21150-21-0
amanullin	H	H	NH_2	OH	$C_{39}H_{54}N_{10}O_{12}S$	21803-57-6
proamanullin	H	H	NH_2	H	$C_{39}H_{54}N_{10}O_{11}S$	54532-46-6
Generic listing for "amanitin"						11030-71-0

2. Synonyms: The formal (Chemical Abstracts) name of α-amanitin is: (cyclic(L)-asparaginyl-4-hydroxy-L-prolyl-(R)-4,5-dihydroxy-L-isoleucyl-6-hydroxy-2-mercapto-L-tryptophylglycyl-L-isoleucyl-glycyl-L-cysteinyl) cyclic (4 → 8)-sulfide(R)-S-oxide). For all amanitins, the name "amanitine" is interchangeable and is used by some authors.

3. Molecular weight: Amanitins crystallize as hydrates; α-amanitin crystallizes as tetrahydrate (mol. wt. 990), β-amanitin as trihydrate (mol. wt. 973), and γ-amanitin as tetrahydrate (mol. wt. 974) (Wieland and Faulstich, 1978).

4. Density: no data.

5. Absorption spectroscopy: All amanitins have the same UV spectrum with a λ_{max} = 303 nm at pH 1-6, with a characteristic bathochromic shift to 330 nm on addition of alkali (pH 12). In both pH ranges, ε = 12,023 (Wieland, 1983; Fürlinger and Wolfbeis, 1983). This pH effect is due to ionization of the aromatic OH group, for which a pK of 9.71 has been calculated. Amanin, which lacks this OH group, has a λ_{max} at 289 nm and shows no bathochromic shift (Gebert et al., 1967). α-Amanitin (and presumably β and γ as well) is strongly phosphorescent at 77°K but shows only weak fluorescence (Fürlinger and Wolfbeis, 1983). The CD spectrum of all amanitins (identical) has been shown (Wieland and Faulstich, 1978).

* δ-amanitin has been separated from other amanitins by paper chromatograph There is no information on chemical structure or toxicity.

- Volatility: no data but may be considered negligible.

- Solubility: Amanitins are readily soluble in water, methanol, liquid ammonia, and pyridine, moderately soluble in ethanol, and insoluble in weakly polar organic solvents. No data for other amatoxins but they probably have the same characteristics.

- Description: Amatoxins are colorless, odorless crystalline compounds.

- Boiling point, melting point: α- and β- amanitin have melting points (with decomposition) of 255 and 300°C, respectively; no data on other amatoxins.

- Stability: There are no data on stability of amatoxins in the solid state; one may assume that they are stable when stored in the dark at refrigerator temperatures. Amatoxins decompose slowly in aqueous solution in open vessels, and rapidly on silica TLC plates when exposed for some hours in daylight and open air (Wieland and Faulstich, 1978; no references or quantitative data for these statements). Heating amatoxins to 100°C for a few minutes does not destroy their activity. There is no known enzyme which degrades them (Wieland and Faulstich, 1978).

- Chemical reactivity: Amanitins act as reducing agents (blue color with Folin-Dennis reagent, yellow-brown with Millon's reagent, and black with ammoniacal silver nitrate). The violet color produced by cinnamaldehyde followed by exposure to HCl vapors is commonly used for visualization on paper and thin layer chromatograms. The phenolic hydroxyl group undergoes the usual reactions (coupling with diazo compounds, etherification, etc.). The sulfoxide group may be reduced, or oxidized to the sulfone. Mild acid splits the molecule to a monocyclic ("seco-") compound. For further discussion see "Structure-Activity Relationships," Section F4b.

- Flash point: not applicable.

- Autoignition temperature: not applicable.

- Explosive limits in air: not applicable.

re, Explosion, and Reactivity Hazards

- Amatoxins are likely to be inactivated under conditions of fire. However, because of their high toxicity on ingestion, it is advisable that fire-fighting personnel wear face masks.

- Flammability is likely to be low. When handled in flammable solvents, the precautions required for such solvents will apply.

- Conditions contributing to instability (and detoxification) are high temperature and acid.

4. No hazardous decomposition products are known.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving amatoxins.

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: No validated method reported.

2. Decontamination: Turn off equipment that could be affected by amatoxins or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Wipe off surfaces with water, then wash with copious quantities of water. Glassware should be rinsed (in a hood) with water, followed by soap and water. Animal cages should be washed with water.

3. Disposal: No waste streams containing amatoxins shall be disposed of in sinks or general refuse. Surplus amatoxins or chemical waste streams contaminated with amatoxins shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing amatoxins shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing amatoxins shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with amatoxins shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing amatoxins shall be handled in accordance with the NIH radioactive waste disposal system.

4. Storage: Store solid amatoxins and their solutions in dark-colored, tightly closed containers under refrigeration.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: no data.

2. Analysis: The most commonly used method for the separation and quantitation of amatoxins in mixtures (e.g., Amanita extracts) is chromatography on columns, paper, and/or silica gel plates, followed by treatment with cinnamaldehyde, exposure to HCl vapors, and densitometry of the resulting violet spots (e.g., Faulstich et al., 1973; Stijve and Seeger, 1979). Far more sensitive bioassays are based either on the highly specific inhibition of RNA polymerase II (Brown and Garrity, 1980) or radioimmunoassay (Fiume et al., 1975; Faulstich et al., 1975). This method has been used in the estimation of amanitins in blood, with a detection limit of 0.5 ng/ml of serum. Neither of these methods distinguishes between toxic amatoxins unless preceded by chromatographic separation. A fluorimetric method, based on reaction of amatoxins (and phallotoxins) with fluorescamine before and after hydrolysis, with a detection limit of 0.1 nmole, has been recently described (Little and Preston, 1984).

Biological Effects (Animal and Man)

1. Absorption: Amatoxins are readily absorbed from the gastrointestinal tract following ingestion by the guinea pig, cat, dog, and man, slowly if at all by the mouse and rat. All species tested exhibit toxic effects on parenteral administration.

2. Distribution: no data.

3. Metabolism and excretion: There appears to be little if any metabolism of amatoxins in intact animals. After intravenous injection of α-amanitin into dogs, the serum level is below detectable levels after 5 hours, and 85% of the applied dose is found in the urine (Faulstich and Fauser, 1973). Similar results were found after intraperitoneal α-amanitin in mice (Fiume et al., 1975). The pathway of amatoxins within the organism shows a great deal of species variation: while excretion of amanitins after parenteral administration is via the kidney and bile, there is reabsorption from the gastrointestinal tract only in the guinea pig, dog, and man (Wieland, 1983), and no renal tubular reabsorption in the rat (Lampe, 1978). These findings probably explain the diversity of toxicities and symptomatologies between mammalian species which is discussed below.

4. Toxic effects: a. Toxicities: The LD50s of amatoxins, administered parenterally, cover a relatively narrow range in a given species (mouse, i.p., in mg/kg: α-amanitin, 0.3; β-amanitin, 0.5; γ-amanitin, 0.2; ε-amanitin, 0.3; amanin, 0.5), except that amanullin and proamanullin are nontoxic at 10 mg/kg (Wieland and Faulstich, 1978). In contrast, there is a wide species variation (α-amanitin, i.p., in mg/kg: guinea pig, 0.05; dog, 0.15; mouse, 0.2-0.3; rat, >2; frog, 15)(Chilton, 1978). The oral LD50 of

α-amanitin in man is estimated to be 0.1 mg/kg. Oral toxicity of amanitins in the mouse and rat is very low because of lack of absorption.

b. Structure-activity relationships: Consideration of the above toxicity data and the chemical structures of amatoxins, shown in Section B1, shows that the only requirement for toxicity is the presence of an -OH group in position R_1. Other chemical modifications of the amatoxin molecule reveal the following (summarized from Wieland and Faulstich, 1978):

i. reduction of the sulfoxide to sulfide, or oxidation to sulfone, has no effect on toxicity.
ii. Reoxidation of the sulfide to sulfoxide results in two stereochemical species, of which only the R-form (which is the form of natural amanitins) is toxicologically active.
iii. acid hydrolysis (see dashed line, structure in Section B1) results in monocyclic, non-toxic "seco-" derivatives.
iv. modification of the phenolic hydroxyl (R_4) by reduction or etherification has no effect on toxicity.
v. conjugation with high molecular weight compounds (serum albumin, γ-globulin, polylysine, dextrans) results in derivatives which are 6-10 times more toxic than the original amatoxin.

c. Symptoms: A distinction must be made in describing toxic symptoms due to administration of amanitins, and those due to ingestion of Amanita phalloides which is the basis of all clinical reports to date. The reason for this distinction is that toxic mushrooms contain considerable amounts of phallotoxins, which, though far less toxic than amatoxins, are fast acting (doses in the neighborhood of their LD100s may produce death in 1-2 hours) and are responsible for all early symptoms of mushroom poisoning (nausea, vomiting, diarrhea). These are observed in all cases of accidental poisoning in man (e.g., Bock et al., 1964; Lampe, 1973; Hatfield and Brady, 1975) and constitute the first phase of such poisoning, appearing within 6-10 hours after ingestion and lasting for a variable length of time. This is followed by a period of remission which can last for several days, during which the patient appears recovered (second phase). The third phase is that which is due to the slow-acting amatoxins and consists of moderate or severe liver and kidney damage, depending on amount of mushrooms ingested (or amanitins administered). These effects have been shown histologically in animals (e.g., Fiume et al., 1969; Marinozzi and Fiume, 1971), using parenteral amanitins, and are mirrored by the usual clinical signs of such poisoning in animals and man (highly increased SGOT and SGPT, icterus, hematuria, and proteinuria), with secondary effects on the heart and central nervous system. Death usually occurs in hepatic coma.

The underlying mechanism for the toxic action of amatoxins is inhibition of protein synthesis in the liver and kidney which is

brought about by the specific inhibition of RNA polymerase II (sometimes called RNA polymerase B). Binding occurs in a 1:1 ratio, and the linkage is a tight one (dissociation constant of the order of 10^{-9} M). When this enzyme is thus inhibited, a new phosphodiester bond can still be formed but translocation of the nascent RNA and enzyme along the DNA template does not occur (for discussion see Campadelli-Fiume, 1978; Wieland, 1983).

5. Carcinogenic effects: No carcinogenic effects of amatoxins have been reported.

6. Mutagenic and teratogenic effects: none have been reported.

G. Emergency Treatment

There is no satisfactory method of treatment of poisoning due to ingestion of toxic mushrooms or parenteral administration of amatoxins; at the time that symptoms appear (or, in the case of mushroom poisoning, are reported), the essential damage has been already done. Ingestion of a slurry of activated charcoal has been proposed and may be of some marginal value in man since some of the poison is reexcreted into the intestine via bile. Cytochrome C in massive doses has increased the survival rate in female mice when given within 30 minutes or a few hours after intraperitoneal α-amanitin (Floersheim, 1972) but this appears to be of no practical importance in human cases of intoxication for the reasons stated above. Hemodialysis, which has been recommended by some authors, is of doubtful value because of rapid clearance of amanitins from blood. Thiooctic (α-lipoic) acid treatment has been proposed, but results are conflicting.

Treatment therefore is mainly symptomatic and supportive, and includes meperidine for pain and cautious intravenous replacement of fluids should be followed by monitoring serum transaminases, blood electrolytes and glucose, and liver and kidney function tests. For discussion see Gosselin et al., 1976 and Lampe, 1978.

H. References

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